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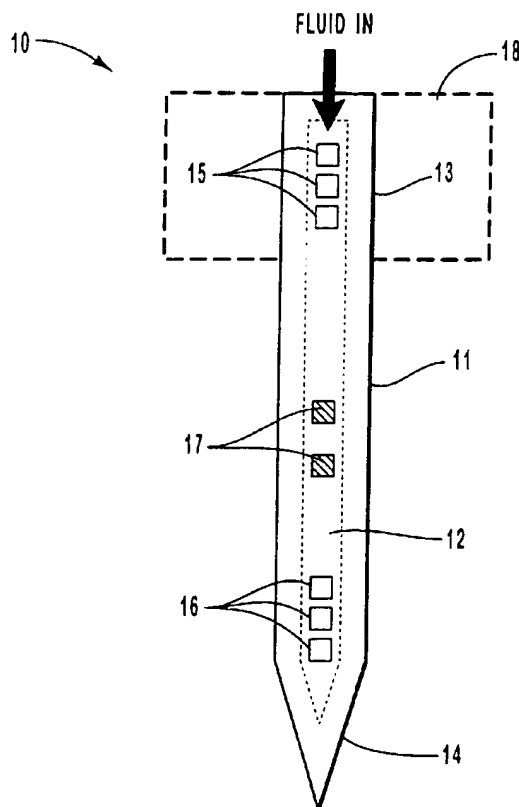
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(54) Title: **ACTIVE NEEDLE DEVICES WITH INTEGRATED FUNCTIONALITY**



(57) Abstract: An active needle device (10) for fluid injection or extraction includes at least one hollow elongated shaft (11) defining at least one channel (12). The channel (12) provides communication between at least one input port (15) and at least one output port (16) of the needle device (10). At least one active component (17) such as a sensor or actuator is placed or integrated into the elongated shaft (11). The needle device (10) can include a macroneedle, a microneedle (21), or an array of macroneedles or microneedles (25a). The microneedles (21) can be fabricated on a substrate (26) which can remain attached to the microneedles (21) or be subsequently removed. The active component can facilitate biochemical, optical, electrical, or physical measurements of a fluid injected or extracted by the needle device (10).

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1 **ACTIVE NEEDLE DEVICES WITH INTEGRATED FUNCTIONALITY**

BACKGROUND OF THE INVENTION

1. **Field of the Invention**

5 The present invention relates generally to needle devices for the injection and extraction of fluids. More specifically, the present invention relates to active needle devices such as macroneedles, microneedles, and macroneedle or microneedle arrays which have one or more active components providing additional functionality to the needle devices.

10 2. **Relevant Technology**

 Micro instrumentation is a rapidly growing area of interest for a broad spectrum of applications. One particularly fast growing area is biomedical instrumentation where significant efforts are being made to develop micro biochemical analysis systems, physiological analysis systems, and drug delivery systems. A variety of manufacturing technologies are used to fabricate these micro systems, many of which are categorized under the set of technologies known as micromachining. The number of biomedical applications for micromachining technologies is rapidly growing. Since micromachining technologies are relatively new, there is an increasing set of manufacturing techniques and critical applications still to be addressed.

20 It is well known that needles are used to extract samples of substances from living tissue in order to analyze the substances for diagnostic purposes, or to deliver a drug or medicament. The majority of needles in use today are macroneedles, which have a relatively large diameter as compared to a blood cell and are on the order of millimeters (mm).

25 In many areas of biotechnology and medicine, there exists the need for fluid injection or extraction on a microscale; either for injection into a precise location, or for injecting or extracting small amounts of fluid. It is advantageous to be able to perform these injections or extractions with a minimal amount of tissue damage, and also with a minimum amount of discomfort and pain to patients. Microneedles and microneedle arrays are capable of performing these tasks. For example, microneedles and microneedle arrays can be used as precise injection/extraction needles in cell biology, as injection/extraction heads in a drug delivery system or microchemical

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1 factory, and as injection/extraction heads in microsurgery. Some of the smallest
hollow needles that are currently available have inner diameters of over 200 μm .
Prior micro-sized (sizes on the order of microns, where 1 micron = 1 μm = 10^{-6}m)
needles include those disclosed in U.S. Patent No. 5,457,041 to Ginaven et al., and
5 U.S. Patent No. 5,591,139 to Lin et al.

For some applications, it is desirable to inject small amounts of fluid;
however, in other situations, larger amounts of fluid are required to be injected. Most
of the prior systems do not have the capability to transmit larger amounts of fluid into
a precise location. One of the methods used to address this problem is to fabricate an
10 array of needles, as in U.S. Patent No. 5,457,041 referred to hereinabove, which
discloses an array of microneedles of about 20 needles by 20 needles, wherein the
length of the needles is between 10 and 25 microns, and the spacing between needles
is between about 5 and 20 microns.

15 In U.S. Patent No. 5,591,139 referred to above, silicon-based microneedles are
disclosed which are fabricated using integrated circuit processes. Various devices
such as microheaters, microdetectors, and other devices can be fabricated on the
microneedle.

Problems with prior microneedles include relatively poor mechanical
durability. This is mainly due to the fact that such microneedles have been made out
20 of etched silicon or out of chemical vapor deposited polysilicon, both of which have a
tendency to be brittle and break easily.

In some cases, it is desirable to analyze the fluids being injected or extracted
by the microneedle(s). Prior microneedles generally have been separate from the
systems used to analyze (chemically, optically, or otherwise) the fluids. In these
25 cases, having a separate analysis system can require additional equipment and is
costly, complex, and inconvenient.

It would therefore be of substantial interest to develop a durable needle device
which is capable of injecting or extracting precise quantities of fluids into specific
locations with a minimal amount of tissue damage, and which has integrated sensing
30 capabilities.

SUMMARY OF THE INVENTION

Active needle devices of the present invention have integrated functionalities

1 such as biochemical, electrical, or optical sensing capabilities. The active needle devices can be active macroneedles or microneedles, as well as active macroneedle or microneedle arrays, which incorporate biosensors therein such as for monitoring metabolic levels.

5 In one embodiment of the invention, an active needle device for fluid injection or extraction includes at least one hollow elongated shaft defining at least one channel therethrough. The channel provides communication between at least one input port and at least one output port of the needle device. At least one active component such as a sensor or actuator is placed or integrated into the elongated shaft. The needle
10 device can be a macroneedle, a microneedle, or an array of macroneedles or microneedles. The microneedles can be fabricated on a substrate which can remain attached to the microneedles or can be subsequently removed. The active component can facilitate biochemical, optical, electrical, or physical measurements of a fluid injected or extracted by the needle device.

15 Two- or three-dimensional microneedle arrays can be constructed having cross-coupling flow channels that allow for pressure equalization, and balance of fluid flow within the microneedle arrays. A plurality of mechanical support members can be integrated into the arrays for stability and to control the penetration depth of the microneedles. In addition, an active microneedle or microneedle array may include
20 various functionalities such as integrated biochemical sensors, as well as electrical, optical, or mechanical transducers and sensors. The sensors reduce or eliminate the requirement for additional external analysis equipment and enhance overall device portability, disposability, and compactness.

25 The active microneedle and microneedle arrays of the invention are particularly useful for fluid extraction and analysis. In one embodiment, an active microneedle or microneedle array includes biochemical sensing reagents which are deposited on an inner surface of the microneedle(s), with a window provided on a surface of the microneedle(s) for the detection of bioluminescence which occurs in the presence of certain metabolic substances.

30 A method of fabricating an active microneedle device according to the present invention includes providing a substrate with a substantially planar surface and depositing a metal material on the planar surface to form one or more bottom walls

1 for one or more microneedles. A top surface of the bottom walls is coated with a photoresist layer to a height corresponding to a selected inner height of a microchannel for each of the microneedles. A metal material is then deposited to form side walls and a top wall upon the bottom walls and around the photoresist layer.
5 The photoresist layer is then removed from each microchannel to form the microneedles. The microneedles can be released from the substrate and used independently of the substrate, if desired.

The method of fabricating the active microneedle device can include p⁺ etch-stop membrane technology, anisotropic etching of silicon in potassium hydroxide,
10 sacrificial thick photoresist micromolding technology, and micro-electrodeposition technology. For a biochemical luminescence sensing microneedle device, certain biochemical sensing reagents can be drawn into and dried onto the inner walls of the hollow needle(s).

The active needle devices of the invention have the advantage of permitting
15 real-time analysis of a fluid being sampled. In addition, the array of active microneedles are capable of injecting or extracting relatively large quantities of fluids with minimal tissue damage and can be readily attached to a standard syringe. The array of active microneedles or a single active microneedle can also be easily and economically fabricated. Yet another advantage of the array of active microneedles
20 or a single active microneedle is a high degree of mechanical durability.

These and other features of the present invention will become more fully apparent from the following description, or may be learned by the practice of the invention as set forth hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

25 In order to more fully understand the manner in which the above recited and other advantages of the invention are obtained, a more particular description of the invention briefly described above will be rendered by reference to specific embodiments thereof which are illustrated in the appended drawings. Understanding that these drawings depict only typical embodiments of the invention and are not
30 therefore to be considered limiting of its scope, the invention will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

1 Figure 1A is a schematic representation of a single active needle device according to the present invention;

 Figure 1B is a schematic representation of a portion of a single active needle device in the form of a microneedle according to one embodiment of the present invention;

5 Figures 2A and 2B are schematic depictions of multi-lumen active microneedles according to alternative embodiments of the present invention;

 Figure 3 is a schematic representation of microneedles in a two-dimensional array according to a further embodiment of the present invention;

10 Figure 4 is a schematic representation of microneedles in a two-dimensional array according to another embodiment of the present invention;

 Figure 5 is a schematic representation of microneedles in a two-dimensional array according to another embodiment of the present invention;

15 Figures 6A-6B are schematic depictions of single-lumen active microneedle arrays according to alternative embodiments of the present invention;

 Figures 7A-7B are schematic depictions of multi-lumen active microneedle arrays according to alternative embodiments of the present invention;

 Figures 8A-8F schematically depict the fabrication process sequence for forming a microneedle array;

20 Figure 8G depicts an embodiment of a multilumen microneedle formed according to the present invention;

 Figures 9A and 9B depict alternative methods of assembling two-dimensional needle arrays into three-dimensional needle array devices;

25 Figure 10 is a schematic depiction of a pressure sensor in an active microneedle according to the invention;

 Figure 11 is a graph of the normalized bioluminescent signal as a function of glucose concentration;

 Figure 12 is a graph of the normalized bioluminescent signal as a function of time for several different concentrations of creatine;

30 Figure 13 is a graph of the normalized bioluminescent signal as a function of creatine concentration; and

 Figure 14 is a graph of the average CCD counts as a function of volume for

1 different sample volumes.

DETAILED DESCRIPTION OF THE INVENTION

5 The present invention is directed to active needle devices, such as macroneedles, microneedles, and macroneedle or microneedle arrays, which have one or more active components incorporated therein providing additional functionality to the needle devices. An active needle device according to the invention generally includes at least one hollow elongated shaft defining at least one channel therethrough. The channel provides communication between at least one input port and at least one output port of the needle device. At least one active component such as a sensor or actuator is placed or integrated into the elongated shaft. The active component can facilitate biochemical, optical, electrical, or physical measurements of a fluid injected or extracted by the needle device. The active component also reduces or eliminates the requirement for additional external analysis equipment, and enhances the overall device portability, disposability, and compactness.

15 Referring to the drawings, wherein like structures are provided with like reference designations, Figure 1A is a schematic representation of a single active needle device 10 according to one embodiment of the present invention. The needle device 10 includes a hollow elongated shaft 11 defining a single channel 12 from a proximal inlet end 13 to a distal tip end 14 which is tapered. One or more input ports 20 15 are located at the proximal inlet end 13 of elongated shaft 11 and one or more output ports 16 are located toward the distal tip end 14. The channel 12 provides communication between input ports 15 and output ports 16.

25 The needle device 10 can be either a macroneedle or a microneedle, depending on the configuration of elongated shaft 11 defining channel 12. A "macroneedle" as used herein means any conventional type needle used to inject or extract fluids, such as conventional metallic needles used in medical applications. A "microneedle" as used herein means any micro-sized needle which can be generally fabricated by micromachining techniques.

30 At least one active component 17 is placed or integrated directly in elongated shaft 11. The active component 17 can include one or more electronic components, electromechanical components, mechanical components, biochemical components, or various combinations thereof. These components can be configured to form one or

1 more sensors, actuators, or combinations thereof. For example, the sensors can be a
biosensor, such as a bioluminescence-based biosensor, which is discussed in further
detail hereafter, microdetectors, optical pressure sensors, DNA analyzer chips, and the
like. The actuators can include electromechanical components for fluid manipulation
5 such as micropumps, microvalves, or the like. In addition, active component 17 can
include an integrated circuit chip, an optical detector, other electrical based
components, impedance analyzers (e.g., integrated resistance, inductance, and
capacitance sensors), transducers, and the like. A transducer can be used for detection
of fluid parameters such as flow velocity, temperature, pressure, etc. The active
10 component 17 can facilitate one or more biochemical, optical, electrical, or physical
measurements or other analyzing functions for a fluid injected or extracted by needle
device 10.

The active component 17 can be interfaced electronically with conventional
analyzing equipment or can be a stand alone device. In addition, the active
15 component 17 may be fabricated independently of the needle device and subsequently
placed therein, or may in some cases be fabricated at the same time as the needle
device. If fabricated independently, the active component can be made on separate
chips, which can be affixed to an internal surface of the needle or substituted for part
of a wall of the needle. Additional details related to active component 17, including
20 specific embodiments thereof, are discussed below.

The active component 17 allows the needle device to provide real-time
analysis of a fluid being sampled such as in a blood analysis system. For example,
blood can be analyzed by an on-chip amplifier, such as a blood analysis amplifier, and
an A/D converter which converts an analog signal to a digital signal for transmission
25 to a computer for real-time computer analysis.

When needle device 10 is configured as a single microneedle, elongated shaft
11 generally includes a bottom wall formed by a solid layer 19, and opposing side
walls connected by a top wall which are formed by a hollow layer 20, as shown in
Figure 1B. These bottom, side, and top walls define channel 12 in the form of a
30 microchannel with a transverse cross-sectional profile that is substantially rectangular.
The microneedle optionally has a flange 18 at proximal inlet end 13 (Figure 1A)
which functions as a structural support for the microneedle. The flange 18 can control

1 penetration depth and can be used to mechanically fix the microneedle to a surface
that is penetrated. The input ports 15 located at proximal inlet end 13 and output
ports 16 are formed in one or more of the bottom wall, side walls, or top wall of the
microneedle. The input ports 15 and output ports 16 in the microneedle can be
5 formed by conventional fabrication processes such as etching. Figure 1B shows
active component 17 such as a biosensor adjacent to output ports 16.

The microneedle can be fabricated by micromachining techniques from a
variety of metallic materials such as nickel, copper, gold, silver, platinum, palladium,
titanium, chromium, alloys thereof, and the like, as well as other materials such as
10 silicon, polysilicon, ceramics, glass, carbon black, plastics, composites thereof, etc.
In one embodiment, the microneedle is fabricated from a non-silicon material such as
any of the above metallic materials using the micromachining techniques discussed in
further detail below. The microneedle can be fixed on a substrate where fabricated or
can be removed from a substrate after fabrication.

15 Figures 2A and 2B are schematic depictions of multi-lumen active
microneedles according to alternative embodiments of the present invention. Figure
2A illustrates a dual-lumen active microneedle 21 which is constructed in a similar
manner as the microneedle of Figure 1B, having an elongated shaft 11 including a
bottom wall formed by a solid layer 19, and opposing side walls connected by a top
20 wall which are formed by a hollow layer 20. The hollow layer 20 is configured such
that microneedle 21 has two microchannel members 22a and 22b each with a lumen
therein and each with corresponding inputs 23a and 23b. One or more active
components 17 such as biosensors are located in each of microchannel members 22a
and 22b.

25 Figure 2B illustrates a tri-lumen active microneedle 24 which is constructed in
a similar manner as microneedle 21. The microneedle 24 has an elongated shaft 11
including a bottom wall formed by a solid layer 19, and opposing side walls
connected by a top wall which are formed by a hollow layer 20. The hollow layer 20
is configured such that microneedle 24 has three microchannel members 22a, 22b, and
30 22c therein, each with corresponding inputs 23a, 23b, and 23c. One or more active
components 17 such as biosensors are located in each of microchannel members 22a,
22b, and 22c.

1 The multi-lumen active microneedles according to the present invention allow two or more different fluids to be delivered by the microneedle, which are mixed just before delivery. The multi-lumen active microneedles can also be used to separately distribute an extracted fluid for multiple analyses.

5 Figure 3 is a schematic depiction of an active microneedle array 25a according to another embodiment of the invention. The microneedle array 25a is formed in a two-dimensional configuration on a substrate 26 having a substantially planar upper surface 27. The substrate 26 is preferably composed of a semiconductor material such as silicon, although other materials can be employed such as glass,
10 metals, ceramics, plastics, and composites or combinations thereof.

 A plurality of hollow microneedles 28 are formed on upper surface 27 of substrate 26. The microneedles 28 each have a bottom wall, two opposing side walls, and a top wall that define a microchannel therein. Each bottom wall is formed partially on upper surface 27 of substrate 26. The microneedles 28 each have one or
15 more input ports 15 at a proximal end thereof. A distal portion of each microneedle 28 including the microchannels extends beyond an edge of upper surface 27 of substrate 26 in a cantilevered manner and terminates in needle tips 29 which can have a channel opening therein. In addition, one or more outlet ports 16 can be located adjacent to needle tips 29. The microneedles 28 are preferably aligned substantially
20 parallel to each other on substrate 26. One or more active components 17, such as biosensors or other sensing or actuating devices as discussed above, are located in each of microneedles 28.

 The microchannels in the microneedles 28 are preferably dimensioned to have a width between sidewalls of less than about 100 μm , and more preferably about 0 μm
25 to about 50 μm . When the width is zero between the sidewalls, the microneedles 28 effectively become one multilumen microneedle with a plurality of microchannels, as shown in Figures 2A and 2B. The height between the top and bottom walls of the microchannel is also preferably less than about 100 μm , and more preferably about 2 μm to about 50 μm . The length of each microneedle can be from about 0.05 μm to about 5 mm, and the width of each microneedle can be from about 0.05 μm to about 1 mm. The center-to-center spacing between individual microneedles can be from
30 about 50 μm to about 200 μm . The microneedles can also withstand flow rates of up

1 to about 1.5 cc/sec.

The microneedle length extended from substrate 26 can be varied from less than about 50 μm (subcutaneous) to several millimeters for fluid delivery/extraction. For example, the distal end of each microneedle 28 can extend beyond the edge of substrate 26 a distance from about 10 μm to about 100 mm. The inner cross-sectional dimensions of the microchannels in individual microneedles can range from about 10 μm to about 1 mm in width and from about 2 μm to about 50 μm in height. Accordingly, the microchannel in each of the microneedles can have a cross-sectional area in the range from about 25 μm^2 to about 5000 μm^2 .

10 A needle coupling channel member 30 can be optionally formed on upper surface 27 of substrate 26 between microneedles 28. The coupling channel member 30 has a bottom wall, two opposing side walls, and a top wall that define a coupling microchannel therein, which provides for fluid communication between the microchannels of each microneedle 28. The coupling channel member 30 also allows for pressure equalization and balance of fluid flow between microneedles 28.

15 Structural support members 31 can be formed on either side of coupling channel member 30 on upper surface 27 of substrate 26. The structural support members 31 mechanically interconnect microneedles 28 to provide rigidity and strength to microneedle array 25a. The support members 31 also precisely control the penetration depth of microneedles 28.

20 The microneedles 28, coupling channel member 30, and support members 31 can be formed from a variety of metal materials such as nickel, copper, gold, palladium, titanium, chromium, alloys or combinations thereof, and the like, as well as other materials such as silicon, polysilicon, ceramics, glass, carbon black, plastics, composites or combinations thereof, and the like. The microneedles 28 can be in fluid communication with a single fluid input device or with multiple fluid input devices (not shown).

25 An active microneedle array 25b according to an alternative embodiment of the invention is shown in Figure 4. The microneedle array 25b has a two-dimensional configuration with similar components as microneedle array 25a discussed above, except that the substrate has been removed from the array. Accordingly, microneedle array 25b includes a plurality of microneedles 32 with microchannels that are

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1 dimensioned as discussed above for microneedle array 25a.

The microneedles 32 each terminate at a needle tip 33 with a channel opening 34 therein. A needle coupling channel member 36 with a coupling microchannel therein provides a fluidic interconnection between the microchannels of each microneedle 32. A pair of structural support members 38 are formed on either side of coupling channel member 36 and interconnect with microneedles 32. One or more input ports 37 and output ports 39 can be optionally formed in microneedles 32 to increase fluid input and output flow. The input ports 37 and output ports 39 can be formed in one or more of the bottom walls, side walls, or top walls of microneedles 32 by conventional fabrication processes such as etching. One or more active components 17, such as biosensors or other sensing or actuating devices as discussed above, are located in each of microneedles 32.

An active microneedle array 40 according to another embodiment of the invention is shown in Figure 5. The microneedle array 40 has a two-dimensional configuration with similar components as microneedle array 25b, except that microneedle array 40 is formed without a coupling channel member. Accordingly, microneedle array 40 includes a plurality of microneedles 32 with microchannels that are dimensioned as discussed above for microneedle array 25a. The microneedles 32 each terminate at a needle tip 33 with a channel opening 34 therein. A pair of structural support members 38 interconnect with microneedles 32. One or more input ports 37 and output ports 39 can be optionally formed in microneedles 32 to increase fluid input and output flow. One or more active components 17, such as biosensors or other sensing or actuating devices as discussed above, are located in each of microneedles 32.

25 Figures 6A-6B and 7A-7B are schematic depictions of single and multi-lumen active microneedle arrays according to alternative embodiments of the present invention. An active microneedle array 42a is shown in Figure 6A having a two-dimensional configuration with similar components as microneedle array 25b discussed above. Accordingly, microneedle array 42a includes a plurality of microneedles 32 each with single lumen microchannels 43. The microneedles 32 each terminate at a needle tip 33. A needle coupling channel member 36 with a coupling microchannel 44 therein provides a fluidic interconnection between

1 microchannels 43 in each microneedle 32. A structural support member 38
interconnects microneedles 32. One or more input ports 37 and output ports 39 are
located in microneedles 32. One or more active components 17 are located in each of
microneedles 32.

5 Another active microneedle array 42b is shown in Figure 6B. The
microneedle array 42b has a two-dimensional configuration with similar components
as microneedle array 42a, except that microneedle array 42b is formed without a
coupling channel member. Accordingly, microneedle array 42b includes a plurality
of microneedles 32 each with single lumen microchannels 43. The microneedles 32
10 each terminate at a needle tip 33 and a structural support member 38 interconnects
microneedles 32. One or more input ports 37 and output ports 39 are located in
microneedles 32, and one or more active components 17 are located in each of
microneedles 32.

15 Figure 7A depicts an active microneedle array 45a having a two-dimensional
configuration with similar components as microneedle array 42a discussed above,
except that microneedle array 45a has a duel lumen microchannel configuration.
Accordingly, each microneedle 32 in array 45a is constructed to have a pair of
microchannels 46a and 46b. A needle coupling channel member 36 with a coupling
microchannel 47 therein provides a fluidic interconnection between microchannels
20 46a and 46b in each microneedle 32. One or more input ports 37 and output ports 39
are located in microneedles 32, and one or more active components 17 can be located
in each microchannel of microneedles 32.

25 Figure 7B depicts an active microneedle array 45b having a two-dimensional
configuration with similar components as microneedle array 42b discussed above,
except that microneedle array 45b has a duel lumen microchannel configuration.
Accordingly, each microneedle 32 in array 45b is constructed to have a pair of
microchannels 46a and 46b. A structural support member 38 interconnects
microneedles 32, and one or more input ports 37 and output ports 39 are located in
microneedles 32. One or more active components 17 can be located in each
30 microchannel of microneedles 32.

In other alternative embodiments, active microneedle arrays can be fabricated
to have additional microchannels in each microneedle. For example, each

1 microneedle in the array can have a tri-lumen channel construction such as shown for the microneedle of Figure 2B discussed above.

It should be understood that the above discussion related to microneedle arrays is equally applicable to macroneedles, which can be configured in various arrays. The macroneedle arrays can have similar structures as described above for the microneedle arrays such as single or multi-lumen channel configurations, multiple input and output ports, structural support members, coupling channel members, and active components in the macroneedles.

A method of fabricating a two-dimensional microneedle array according to the invention is depicted schematically in Figures 8A-8F. As shown in Figure 8A, a substrate 26 having a substantially planar surface 27 is provided, such as a silicon wafer which is polished on both sides. The wafer can have a thickness of about 1 μm to about 700 μm , and is preferably about 150 μm thick. One side of the wafer is heavily doped with boron using high temperature thermal diffusion in order to form a 3-5 μm thick p+ layer. Next, silicon nitride (Si_3N_4) is deposited on both sides of the wafer using plasma-enhanced chemical vapor deposition (PECVD). The silicon nitride on the undoped side of the wafer is patterned and etched employing photoresist as a mask, and then isotropic etching (a CF_4 plasma for example) is used to etch the exposed silicon nitride to define the area upon which the microneedles are to be fabricated. After patterning the silicon nitride layer, the exposed silicon is anisotropically etched using a potassium hydroxide (KOH) solution. The p+ boron layer serves as an etch stop, resulting in a thin sacrificial membrane 52, as shown in Figure 8A. The sacrificial membrane 52 comprises the surface upon which the microneedles are formed and then subsequently released as described below.

Next, a metal system of adhesion layers and an electroplating seed layer are deposited (by electron beam evaporation, for example) onto the insulating silicon nitride film. The adhesion and seed layers are typically composed of, but not limited to, titanium, chromium, copper, or combinations thereof. Then, using a mask of the appropriate dimensions and standard photolithographic techniques, this metal multilayer is patterned. A metal material is then electroplated to form one or more bottom walls 54 (e.g., about 20 μm thick) for the microneedles, as shown in Figure 8B. Palladium is one preferred metal for the bottom wall since it provides high

1 mechanical strength and durability, is corrosion resistant, provides biocompatibility
for use in biomedical applications, and is easily electroplated to within precise
dimensions. Other materials which fit the criteria mentioned could also be used
equally well, such as copper, nickel, or gold. When performing the electroplating, the
5 bath chemistry and the electroplating conditions (such as amount of applied current
and time in the electroplating bath) should be precisely controlled for optimum
results. The typical dimensions for bottom walls 54 are about 10-20 μm in thickness,
and about 50 μm wide.

10 After bottom walls 54 have been formed, a commercially available thick
photoresist is deposited (*e.g.*, about 20 μm thick) and patterned using ultraviolet
exposure and alkaline developer, resulting in sacrificial layers 56 as depicted in
Figure 8C. Next, a metal seed layer such as gold is sputter deposited (*e.g.*, about 800
 \AA thick) onto the photoresist sacrificial layers 56. The metal seed layer serves as an
electrical contact for the subsequent metal electroplating.

15 A metal layer such as palladium is then electroplated (*e.g.*, about 20 μm thick)
onto sacrificial layers 56 to form a plurality of side walls 58 and top walls 60 of each
microneedle, as shown in Figure 8D. The exposed metal seed layer is then removed
using wet etching techniques to expose the underlying photoresist. Once the metal
seed layer has been etched, the wafer is placed in an acetone bath to remove the
20 photoresist from inside the microneedle structures, thereby producing a plurality of
hollow microneedles 28, as shown in Figure 8E.

In the final processing step, sacrificial membrane 52 is removed by an
isotropic etching technique, such as reactive ion etching in a SF_6 plasma. Thus, the
microneedle ends are released from sacrificial membrane 52 and are freely suspended,
25 projecting outward from substrate 26, as depicted in Figure 8F and in the embodiment
of Figure 3.

Alternatively, the fabricating method outlined above can be used to form a
multilumen microneedle by forming bottom walls 54 with zero spacing therebetween
on substrate 26 and carrying out the remaining steps as described above. A resulting
30 multilumen microneedle 62 with a plurality of microchannels 64 therein is shown in
Figure 8G.

In an alternative fabrication method, the microneedle array can be released

1 from substrate 26 following surface fabrication on substrate 26, such as for the
embodiment shown in Figure 4. This method does not require sacrificial membrane
formation or KOH etching. Instead, the needle arrays are released using wet etching
of the seed metal from underneath the needle structures. If the seed metal is copper,
5 for example, then this can be done by a selective etch of ammonium hydroxide
saturated with cupric sulfate.

Arrays of two up to hundreds of microneedles can be easily and economically
fabricated in a package with dimensions on the order of millimeters according to the
above procedure. A single microneedle such as shown in Figure 1B can also be
10 fabricated according to the procedure outlined above. In addition, instead of having
the fluid outlet at the tips of the microneedles, fluid outlet ports can be etched into the
side walls, top walls, and/or bottom walls of the microneedles if a larger amount of
fluid transfer is desired.

Using the above fabrication methods provides the flexibility to integrate many
15 additional functions directly onto the microneedles. These fabrication methods are
low temperature and are compatible with integrated circuit (IC) technology as a post
process. For example, various active components such as sensors, transducers, and
electronic interfaces may be fabricated as integrated components on-chip during the
needle fabrication process. An example of a procedure for fabricating a microneedle
20 with on-chip devices such as CMOS (complementary metal-oxide semiconductor)
devices is disclosed in U.S. Patent No. 5,591,139 to Lin et al., the disclosure of which
is incorporated by reference herein. Alternatively, the active components may be in
the form of prefabricated chips which are subsequently placed within the needle
channels.

25 All of the needle devices including microneedles and arrays of the present
invention can be coated on the inside with biocompatible materials, such as silicon
nitride, gold, plastics, etc., by conventional coating processes known to those skilled
in the art.

30 In addition, a light enhancing reflective coating can be formed on the inner
surfaces of the active needle devices defining the lumens therein. The reflective
coating can enhance the light output generated during a bioluminescent reaction in a
bioluminescent-based active needle to provide for more sensitive biosensing

1 capabilities. Suitable reflective materials that can be used to coat the inner surfaces of
the needle devices include silver, chromium, titanium, platinum, combinations
thereof, and the like. Increased light intensity has also been observed for long narrow
5 channels, which can be used to effectively design a bioluminescent-based micro-
needle. Further details related to light enhancement of micro reaction chambers and
long narrow (micro) channels are described in Bartholomeusz et al.,
"BIOLUMINESCENT BASED BIOSENSOR FOR POINT-OF-CARE
DIAGNOSTICS," First Annual International IEEE Conference on Microtechnology,
Medicine, and Biology, Lyon, France, October 12-14, 2000, the disclosure of which is
10 incorporated by reference herein.

Figures 9A and 9B depict alternative methods of assembling two-dimensional
needle arrays into three-dimensional needle array devices. In the method depicted in
Figure 9A, a plurality of two-dimensional microneedle arrays 70 are provided which
have been released from a substrate as shown for the array of Figure 4. The
15 microneedle arrays 70 are positioned in a stacked configuration with a plurality of
metallic spacers 72 therebetween to define the distance between any two microneedle
arrays in the stack. The stacked needle array configuration is then subjected to flash
electroplating to join microneedle arrays 70 with metallic spacers 72 in a fixed three-
dimensional needle array device 74. The needle array device 74 can then be disposed
20 in a machined interface structure 76, such as an acrylic interface, allowing connection
to a dispensing means for injecting a liquid such as a syringe.

In the method depicted in Figure 9B, a plurality of two-dimensional
microneedle arrays 80 are provided on substrates 82 such as shown for the
microneedle array of Figure 3. The microneedle arrays 80 are positioned in a stacked
25 configuration, with substrates 82 acting as spacers between arrays 80, to define the
distance between any two microneedle arrays in the stack. The stacked array
configuration is placed in a mold 84 such as an aluminum mold for plastic injection
molding. The stacked array configuration is then subjected to plastic injection
molding. This bonds microneedle arrays 80 together with a plastic molding material
30 86, thereby forming a fixed three-dimensional needle array device 88. The array
device 88 can then be disposed in an interface structure 76 allowing connection to a
dispensing device such as a syringe.

1 In another alternative method of assembling two-dimensional needle arrays
into three-dimensional needle array devices, the two dimensional arrays are manually
assembled under a microscope. The two-dimensional arrays are stacked with spacers
or with substrates on the arrays and are bonded together with a polymeric adhesive
5 such as a UV-curable adhesive to form a three-dimensional needle array device,
which can then be disposed in an interface structure.

 The fabricated three-dimensional needle array devices are typically
dimensioned to have a length of about 5 mm, a width of about 5 mm, and a height of
about 2 mm.

10 The interface structures for connection to a syringe can be made from a variety
of plastic materials such as acrylics, polystyrene, polyethylene, polypropylene, and
the like. The interface structure typically accommodates a three-dimensional needle
array device having up to about 25 two dimensional arrays. The interface structures
are bonded to the three-dimensional needle array devices with a polymeric adhesive
15 such as a UV-curable adhesive. The interface structures are configured to accept
direct syringe connection via a connection means such as a conventional Luer-lock
connector. Alternatively, interface structures can be formed for a single two-
dimensional array or a single microneedle so as to accept direct syringe connection
via a connector such as a Luer-lock connector.

20 In alternative embodiments of the invention, any of the above described array
embodiments can be fabricated as a slanted active needle array, with needles in the
array having varying lengths. The slanted needle array allows for collection of
interstitial fluid samples at different depths during sample acquisition from a patient.
Additionally, the slanted needle array allows for dispensing of medicaments into a
25 patient at multiple depths throughout and beneath the dermal layers of the skin.

 Further details regarding suitable microneedle and microneedle arrays suitable
for use in the present invention are described in copending application Serial No.
PCT/US99/21509, filed September 17, 1999, entitled "Surface Micromachined
Microneedles" and which is incorporated by reference herein.

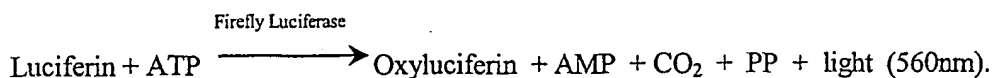
30 As discussed above, one or more active components such as a biosensor can
be placed or integrated directly into the needle devices of the present invention. A
biosensor is basically an analytical device that converts the concentration of an

1 analyte in a sample into an electrical signal by way of a biological sensing element
intimately connected with a transducer. A particularly suitable biosensor useful in the
needle devices of the invention is a bioluminescence-based biosensor, which can be
employed in any of the above described embodiments. Such a biosensor can be
5 formed by depositing biochemical sensing reagents on one or more inner walls of a
needle device. For example, various biosensing reagents can be drawn into and dried
down onto the inner walls of the hollow microneedle or microneedle arrays described
previously. Bioluminescence-based biosensors are particularly suitable for
monitoring metabolic levels, such as the level of creatine or glucose in blood.
10 Generally, bioluminescent detection of the biosensor output is performed at the output
ports of the microneedle.

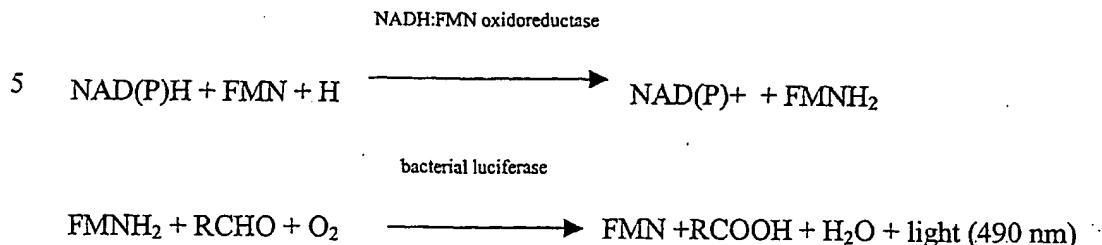
Bioluminescence-based chemical analysis has the advantages of being
substantially more sensitive than conventional chromogenic (absorbance)
measurements and is known to be accurate over a range of five or more orders of
15 magnitude of concentration ranges. It is known that firefly bioluminescence occurs
by enzyme-catalyzed oxidation of luciferin utilizing adenosine triphosphate (ATP).
Bacteria bioluminescence is closely coupled to nicotinamide adenine dinucleotide
hydride (NADH). Bioluminescent-based chemical analysis has the potential of
measuring a wide range of metabolites from smaller sample volumes.

20 Since most metabolites in the body are within one or two reactions from ATP
or NADH, they can be measured by coupling other enzyme reactions to an ATP or
NADH bioluminescent reaction and measuring the light output. During the
production or consumption of a metabolite of interest, enzyme linked reactions will
cause the production or consumption of ATP (or NADH) through the following
25 bioluminescent platform reactions.

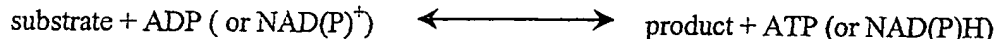
The ATP platform is based on the firefly luciferase reaction:



- 1 The NADH platform reaction based on NADH:FMN oxidoreductase and Bacterial Luciferase:



- 10 Substrates are coupled to these platform reactions through the following generic reaction:



- 15 The detected changes in light intensity (measured by various light detectors as described below) will be stoichiometrically proportional to the time changing concentration of the platform molecule (ATP or NADH) and thus proportional to the metabolite of interest. The bioluminescent platform reactions can be used to measure metabolites such as L-Phenylalanine, D-Glucose, L-Lactate, glucose 6-phosphate, glucose 1-phosphate, UDP-glucose, UDP-galactose, lactose, alkaline phosphatase, galactose in solution, human blood (serum & plasma), sweat, and human milk.

- 20 The light produced in the bioluminescent platform reactions can be detected by an optical detector such as a photodiode or charge coupled device (CCD). Such photosensing devices can be integrated inside a microneedle to detect the light, or can be located externally and can be used to detect the luminescence through a window in a microneedle surface. Depending on the instrument used to detect the luminescence, nanomolar, picomolar or even femtomolar analyses are possible. With increased sensitivity, smaller amounts of sample fluid are required for accurate analysis. A 0.05 micro-liter sample size that can be drawn by the microneedles can painlessly access small amounts of body fluid that can be analyzed via bioluminescence.

- 30 The transmittance of light through a medium across a certain length (l) is specified through Beer's law, $T = 10^{-\text{Absorbance}}$, where $\text{Absorbance} = \epsilon_\lambda C_B (l-x)$, and ϵ_λ is the molar absorption coefficient in $\text{dm}^3/\text{mole-cm}$, and C_B is the concentration in mole/dm^3 . Assuming the light travels through a homogeneous medium along length

1 mole/dm³. Assuming the light travels through a homogeneous medium along length
(*l*), the total transmitted light (*T*) can be expressed as:

$$T_{total(l)} = \int_{length} 10^{-\epsilon_{\lambda} C_B (l-x)} dx = (1-10^{-l}) / L n 10$$

5 where $\epsilon_{\lambda} C_B$ is assumed to be 1 for simplicity. For a pyramid (which is the shape of
the etched wells of the <100> silicon wafers), where the peak depth of the pyramid is
equal to the length (*l*) and the base width of the pyramid is (*a*), the transmittance is
integrated through the viewing area and is equal to $T_{total(l)} \cdot a^2/3$. Thus, a linear
correlation of a volume versus integrated luminosity plot for a given viewing window
should indicate a transmittance proportional to signal intensity (average CCD counts
10 over the viewing area, for example). A flat bottom well with a reflective surface will
have a transmittance of $T_{total(2l)}$, and will be about twice as large as $T_{total(l)}$ (when length
<< 1^{cm}). Thus, the slope of a linear fit of volume versus integrated luminescence
intensity for a reflective surface of a given viewing window should be about twice
that of a non-reflective surface due to the increased transmittance.

15 The enzymes suitable for use in the bioluminescent platform reactions of the
biosensors are inherently robust and stable, retaining their activity after lyophilization,
deposition, and rehydration. The reagent/enzyme mixture or "cocktail" used to detect
various metabolites is drawn into the needles after fabrication, lyophilized (freeze
dried) in a stable state, and then packaged for long term storage (up to a year
20 depending on the analytes, reagents, and enzymes needed). The reagent/enzyme
mixture includes the ATP and NADH bioluminescent platform enzymes and the
enzymes that couple the metabolites to the platforms, as well as all the necessary
reagents needed to complete all the reactions that lead to the light output.

25 The methods used for preparing and storing reagent/enzyme mixtures inside
the bioluminescent-based active needle devices are discussed as follows.

1. Drawing of Reagent/Enzyme Mixtures into the Needle Lumen(s)

The reagent/enzyme mixtures or "cocktails" are prepared as a "wet" solution
in the concentrations needed to detect the desired analytes from the sample volume
drawn through the needle lumen. Anti-oxidants and other enzyme stabilizing agents
30 are added to the "cocktail" before lyophilization. These enzyme-stabilizing agents are
described below in the lyophilization process.

1 The prepared wet solutions are drawn into the needles by capillary action.
The filling of the needles is ensured by oxidizing the lumen of the needle during the
needle fabrication process (or at least using a hydrophilic metal for the lumen of the
needle). Each needle lumen has a different "cocktail" for measuring different
5 analytes. Reagents and enzyme-stabilizing agents can be added to an agarose solution
which is dried during the lyophilization process and allows for the sample fluid to be
drawn into the needle faster by capillary action.

2. Lyophilization Process

10 Lyophilization stabilizes enzymes for long-term storage by reducing both
mechanical and chemical degradation. Appropriate stabilizing excipients and
preservatives minimize the denaturation often observed during the processes of
lyophilization. Optimized process variables include the initial concentration of
enzyme, buffers that exhibit minimal changes in pH with freezing and drying,
freezing rate (should be slow), and various additives.

15 The glass transition temperature of the amorphous phase of the lyophilized
enzyme needs to exceed the planned storage temperature of the needle devices. Glass
transition temperature of the lyophilized enzyme can be increased by using
disaccharides and polymers (used in combination with disaccharides). Disaccharides
such as sucrose and trehalose are especially good at stabilizing the enzyme during
20 freezing and dehydration. The sugar to protein weight ratio should be at least about 1
to 1, although stability can be further increased with greater sugar (5 to 1 ratio).
Reducing sugars such as glucose, lactose, maltose or maltodextrins should be avoided
because of their tendency to degrade proteins through the Maillard reaction between
the carbonyls of the sugar and the free amino groups of the protein. Furthermore,
25 surfactants can be used to inhibit aggregation at very low concentrations, such as less
than about 0.5% per volume.

 Antioxidants such as dithiothreitol and glutathione are used during
lyophilization and subsequent storage to prevent oxidation of firefly luciferase due to
sulfhydryl groups. Bovine serum albumin can be used for surface passivation.
30 Polyethylene oxide (PEO)-based polymers and surfactants are also effective for
surface passivation. Various enzyme preservation and stabilization cocktails are well
known and widely used to maintain enzyme activity under dry storage conditions. In

1 one embodiment of the invention, bioluminescent reagents are preserved and
stabilized with the following components: (1) 0.45 M glycyl glycine buffer (pH 7.8);
(2) 1 mM EDTA; (3) 1mM dithiothreitol; (4) 10 mM MgSO₄; (5) 1 mg/ml of bovine
serum albumin; (6) 1 wt% sucrose; and (7) 1 wt% Dextran T-40. It has been found
5 that such a mixture is suitable for stabilization of creatine kinase, a particularly
delicate enzyme.

The actual bioluminescent molecules (ATP, FMN, bacterial and firefly
luciferase, Oxidoreductase, etc.) are added to the preservative reagents, mixed
thoroughly and added to each channel of a pre-chilled biosensor. In one preferred
10 method, the biosensor and reagents are rapidly frozen to about -70°C followed by a
two-stage lyophilization process. The first stage of lyophilization proceeds for 24
hours at about -50°C and less than about 100 mTorr of pressure. The second stage of
lyophilization proceeds for an additional 24 hours at about +30°C and less than about
100 mTorr of pressure. Air is then re-admitted to the lyophilization chamber and the
15 biosensors are removed. Each completed biosensor is then stored in a black plastic
container with a gas tight lid that also contains a desiccant and a humidity indicator
membrane. It has been found that this method used with firefly luciferase can
preserve more than half the enzymatic bioluminescent activity for a minimum of six
months.

20 The interaction between histidine and Ni⁺⁺ can also be used for immobilization
of the enzyme. In biosensor applications, enzymes can be immobilized on a solid
support to prevent diffusion (into the sample solution) and minimize interference with
other channels of the sensor. The performance of the sensor can be adjusted by
changing the immobilized enzyme amount. The recombinant enzymes with BCCP
25 domains can be immobilized through this interaction with high affinity ($K_a=10^{15} \text{ M}^{-1}$).
There are a variety of solid matrices that can be used for immobilization.

Co-immobilization of sequentially operating enzymes improves total reaction
efficiency, leading to higher sensitivity. A coupled assay with coimmobilized
luciferase and flavin reductase has the advantage that the FMNH₂ produced from the
30 flavin reductase can be used more effectively for the luciferase reaction, reducing its
autooxidation. Traditional immobilization methods use chemically conjugated
enzymes on solid materials, resulting in low immobilization efficiency and low and

1 inconsistent enzyme activity, due to nonspecific immobilization and surface-induced activity loss.

In one method of the invention, a biotin-avidin system for protein immobilization is utilized, due to its high affinity ($K_a=10^{15}/M$) and stability. In another method, *V. harveyi* luciferase and FRase I biotinylated is produced in vivo, the proteins are immobilized on avidin-conjugated beads, and the enzyme beads are used to assay NADH. In using this method, it has been found that the co-immobilized enzymes had eight times higher bioluminescence activity than the free enzymes at low enzyme concentration and high NADH concentration. In addition, the immobilized enzymes were more stable than the free enzymes. This immobilization method is also useful to control enzyme orientation, which can increase the efficiency of sequentially operating enzymes like the oxidoreductase-luciferase system.

3. Storage of Active Needle Devices

Storing the bioluminescent-based active needle sensors with the lyophilized "cocktail" in a desiccant will ensure that glass transition temperatures of the lyophilized enzyme are greater than storage temperatures. The active needle devices should be stored in the dark to minimize photooxidation.

Further details regarding bioluminescence-based biosensors as used in the present invention are set forth in the Examples section below.

Another sensor that can be utilized in the needle device of the invention is an integrated pressure sensor in the form of an optical Fabry-Perot cavity, which is depicted in Figure 10. The pressure sensor can be integrated into any of the above embodiments by forming a glass plate 120 on one interior surface 121 of a hollow microneedle. A cavity 125 is etched in glass plate 120 on the side of the sensor which is to be in proximity to the fluid flowing in the microchannel of the needle. A thin silicon diaphragm 126 (*e.g.*, about 25 microns thick) is affixed over cavity 125 and is configured to be in direct contact with a fluid whose pressure is to be measured. The diaphragm 126 deflects in response to applied pressure. A light beam 129 (*e.g.*, produced by an LED) is coupled into the sensor through optical fiber 128. The optical fiber 128 optically communicates with the interior of the microneedle through a window 124 in the side of the microneedle. Pressure changes from fluid flowing past diaphragm 126 cause the diaphragm to move. The cavity 125 functions as a Fabry-

1 Perot cavity and the diaphragm reflects varying amounts of light intensity which is
directly proportional to the amount of pressure applied to the diaphragm by the fluid.
The reflected light can be coupled as a signal out 130 through optical fiber 128 to an
appropriate analyzing device for measurement. The change in reflected light can be
5 measured and with proper calibration, can provide a linear measurement of the
amount of pressure in the fluid.

The active needle devices of the invention such as the microneedle and
microneedle arrays have many benefits and advantages. For fluid injection, these
benefits include reduced trauma at penetration sites due to their small size, greater
10 freedom of patient movement because of the minimal penetration depth of the
needles, a practically pain-free drug delivery due to the smaller cross section of the
needle tip and distribution of fluid force, and precise control of penetration depth from
needle extension length. In addition, the microneedles have the ability to deliver
drugs to localized areas, or extract fluids from precise locations for analysis, and are
15 advantageous in their ability to be stacked and packaged into a three-dimensional
device for fluid transfer. The active microneedles have the ability to extract either
large or small amounts of fluids in precisely controlled amounts, and can be easily
and economically fabricated using micromachining procedures.

The active microneedles also provide convenient and easy methods for
20 performing electrical, optical, physical, or biochemical sensing in compact devices.
The integrated sensing capabilities in the active microneedles provide compact,
portable, and disposable devices which are less costly and complex to use for analysis
of fluid samples, and reduce or eliminate the need for supporting fluidic and
electronic systems.

25 The microneedles can be used in a wide variety of biomedical applications.
The microneedles can withstand typical handling and can subcutaneously deliver
medication without the usual discomfort associated with conventional needles. The
microneedles are minimally invasive, in that the microneedles only penetrate just
beyond the viable epidermis, reaching the capillaries and minimizing the chance of
30 encountering and damaging the nerves present in the area of penetration.

The following examples are given to illustrate the present invention, and are
not intended to limit the scope of the invention.

1

Example 1

A two dimensional microneedle array was fabricated with 25 hollow microneedles fluidly interconnected by a needle coupling channel. The hollow microneedles were made such that their inner cross-sectional area was $40 \times 20 \mu\text{m}^2$ (width by height) and their outer cross-sectional area was $80 \times 60 \mu\text{m}^2$. The needle coupling channel was $100 \mu\text{m}$ wide, and provided fluid communication between each needle channel. Two sets of $60 \times 100 \mu\text{m}^2$ structural supports were located $250 \mu\text{m}$ from each needle end. Each needle channel was 2 mm long, while the width of the needle array was 5.2 mm . The center-to-center spacing between individual needles was $200 \mu\text{m}$. The needle walls were made of electroformed metal and were approximately $20 \mu\text{m}$ thick.

The microneedle array was fabricated from electroformed low stress nickel sulphamate, gold cyanide, and palladium electroforming solutions. The bath chemistry and electroplating conditions were selected and precisely controlled to allow formation of low stress depositions on top of a $3\text{-}5 \mu\text{m}$ sacrificial membrane. The surface roughness of the electroplated metals was found by Atomic Force Microscope (AFM) to be approximately 15 nm , resulting in a relative roughness of 0.00056 .

It is important to note the structural quality of the needle tips. The inner dimensions were approximately $30 \times 20 \mu\text{m}^2$, outer dimensions were approximately $80 \times 60 \mu\text{m}^2$, and the needle tips were formed with 45° angles for ease of penetration.

Example 2

25

Individual hollow metallic microneedles were fabricated with multiple output ports and had an inner cross-sectional area of $140 \times 21 \mu\text{m}^2$ (width by height) with outer cross-sectional area dimensions of $200 \times 60 \mu\text{m}^2$. The tip dimensions of the microneedles were less than $15 \times 15 \mu\text{m}^2$. The length of the tapered portion of the needle shaft was 1 mm and the distance from the tip to the first output port was about $300 \mu\text{m}$. The total length of each microneedle was 6 mm , with input port inner dimensions of $140 \mu\text{m}$ wide and $21 \mu\text{m}$ high. The wall thickness of each microneedle

1 was about 20 μm , and the output ports were formed with dimensions of 30 μm^2 on the top and bottom of the microneedles. The output ports were separated by 30 μm , and there were 9 ports on the top and 12 ports on the bottom of each microneedle.

5 The microneedles were each packaged into a standard Luer-lock fitting using a polymeric medical grade UV-curable adhesive. This interface between a microneedle and a syringe included a simple female luer-to-1/16" barb adapter. The UV-curable adhesive was found to permanently affix the microneedle to the interface while providing a leak resistant seal.

10

Example 3

A preliminary experiment was conducted to assay a glucose via glucokinase and firefly luciferase reactions. The assay was a homogeneous ATP depletion type assay with initial concentrations of glucokinase (80 nano-molar), ATP (10 micro-molar), luciferein (100 micro-molar), and firefly luciferase (0.1 nano-molar) in 0.45 M glycyl glycine buffer at pH 7.8. The results are shown in the graph of Figure 11, which plots the normalized luminometer bioluminescence, detected two minutes after the rapid mixing of all reactants, as a function of initial glucose concentration. The results suggest that a single step homogeneous assay for glucose can be accomplished without initial dilution of the glucose in the sample.

20

Example 4

Figures 12 and 13 are graphs showing the characteristics for a creatine biosensor. The graph of Figure 12 plots the normalized bioluminescent signal (in relative light units) as a function of time for several different concentrations of creatine. The bioluminescent signal had a 560 nm wavelength and was measured by a luminometer. The final concentrations of creatine shown are within the physiological serum creatine concentration range.

25

Figure 13 shows the results of an assay experiment for creatine using the creatine kinase and firefly luciferase reactions. The creatine assay was a homogeneous ATP depletion assay (the creatine kinase reaction competes with the firefly luciferase reaction for the ATP). The final assay constituents were buffered in 0.45 molar glycine-glycine buffer at pH 7.8 and the final assay concentrations were

30

1 the following: 1.4 μM ATP , 71 μM Mg^{2+} , 14 μM luciferin, 0.3 μM luciferase, 60
2 μM creatine kinase, and various concentrations of creatine (0.45 M glycine-glycine
3 buffer for control). Relative light units (RLU) measured by the luminometer are
4 dependent on the type of instrument, sensitivity of the instrument, and other factors;
5 therefore, the raw bioluminescence signal measured versus time was normalized to
6 the control peak RLU value. Figure 13 plots the normalized bioluminescent signal as
7 a function of creatine concentration.

Example 5

10 In order to determine the feasibility and the physical limitations of using
11 bioluminescence for highly sensitive analyte measurement of small sample volumes,
12 micro- reaction chambers (μRCs) were fabricated on silicon wafers using KOH
13 anisotropic wet etching. An ATP firefly luciferase/luciferin solution was placed in
14 the μRCs and observed through a close up lens with a CCD. The integrated CCD
15 signal was recorded and compared with well size and depth. The attenuation of the
16 CCD signal was also observed for wafers coated with titanium (500Å) followed by
17 chromium (1500 Å).

18 A 5-mL firefly luciferase/luciferin solution consisted of 1.25 mg/mL bovine
19 serum albumin (Sigma - reconstituted into the solution and used for coating the glass
20 vial to prevent denaturing of the luciferase), 1.25 mM ethylene diaminetetra acetic
21 acid (Sigma), 12.5 mM Mg^{++} (Sigma - from MgSO_4), 1.84- μM firefly luciferase
22 (Promega), and 1.25 mM luciferin (Biosynth) in a 1.25 mM glycyl-glycine buffer.
23 This mixture was able to maintain 90% activity for about 20 hours when stored in the
24 dark. A 5 mM ATP, glycyl-glycine buffer solution was also prepared. A 20 μL
25 sample of the firefly luciferase/luciferin solution was pipetted into 5 μL of the ATP
26 solution that resulted in a 1.0 mM ATP mixture, saturated with luciferase and
27 luciferin (which means the reaction rate was at its peak). After the solution mixed, 20
28 μL of it was pipetted onto an area about 20 x 15 mm wide. A thin glass cover slip
29 was placed on the solution, starting at one end and tilting the cover slip as it was laid
30 down, so that the excess bioluminescent fluid would disperse. For high
31 concentrations of ATP ($> 8 \mu\text{M}$), the initial mixing of ATP with the
32 luciferase/luciferin solution causes a peak luminescence within 3 seconds. The

1 luminescence then tapers and levels off after 1 minute. Therefore, the light
measurements for this experiment were integrated for 20 seconds, 2 minutes after the
ATP and luciferase/luciferin solution were mixed, with the light intensity essentially
constant.

5 An ST6-A CCD camera, from Santa Barbara Instruments Group was fitted
with an Olympus wide-angle lens and close-up ring. The experimental substrates
were focused 55 mm below the lens with the aperture set at 2.8. The field of view
was about 20 x 15 mm. The camera was operated at -20.00 °C. One minute after the
luciferase/luciferin solution was added to the ATP, a 10-sec dark field exposure was
10 taken with the CCD. At 2 minutes and 5 seconds after the ATP and
luciferase/luciferin solution mixed, the CCD integrated a 20 second exposure of the
bioluminescent reaction while shrouded in a darkroom. The resulting image was
saved as a TIF file (Range: 0-400). Scion Image (based on the NIH image software)
was used to determine the average and standard deviation of the pixel values for each
15 μ RC. One pixel of the CCD image was equal to 46.875 μ m. Actual results failed to
show light emitting from the 75, 50, 25, and 10 μ m wide wells on all substrates.

Figure 14 is a graph of the average CCD counts from the 20 second integrated
CCD reading as a function of different sample volumes. The data was plotted in sets
for the same viewing area (or μ RC square width). The data was also separated
20 according to Ti/Cr reflective substrates and plain, non-reflective substrates. For each
set of data (wells with same width and coating), the increasing volume occurs from
the increased etch depths of 250 μ m, 100 μ m, and 50 μ m. The data from the different
etch depths are pointed out for the 750 μ m wide wells as an example. The higher
intensity values occurred for the deeper wells. The intensity/volumes slope is greater
25 for the Ti/Cr reflective substrates than for the plain substrates.

Table 1 below sets forth a statistical comparison of the intensity/volume slope
relationships for reflective vs. non-reflective surfaces of the same viewing window
size. The reflective wells with intensity/volume slopes that were statistically different
from the intensity/volume slopes for the non-reflective surfaces ($P < 0.05$ using the T-
30 test) were over two times greater. This follows the hypothesis that transmittance is
nearly doubled for chromium coated (reflectivity = 0.67 @ 560 nm) versus uncoated
silicon (reflectivity = 0.33 @ 560 nm) μ RCs.

1 The T-test used was the student-T test to find if there was a significant
 difference between sample measurements of two groups. The null hypothesis of the
 T-test (for the described experiment of this example) was: there is no difference in the
 intensity/volume slopes for the wells with reflective surfaces and the wells with non-
 5 reflective surfaces. The value $P < 0.05$ means that there is less than a 5% probability
 (P) that the null hypothesis is true. The T-test, if performed properly, is a useful tool
 to show that one method or product is significantly different (more efficient, better,
 etc.) than an existing method or product.

10

Table 1

| μ RC Width (μ m) | P value from T-test between Intensity/Volume Slopes of Non-Reflective μ RCs and Reflective μ RCs | Ratio of Intensity/Volume Slopes for Reflective and Non-Reflective Substrates |
|---------------------------|---|---|
| 750 | 0.00001 | 3.02 |
| 500 | 0.002 | 2.37 |
| 400 | 0.004 | 4.94 |
| 300 | 0.005 | 5.11 |
| 250 | 0.36 | 2.87 |
| 200 | 0.030 | 1.07 |
| 150 | 0.72 | 0.88 |
| 100 | 0.55 | 0.21 |

20

The intensity/volume slope also increases as the μ RC square width decreases.
 This implies that using long, narrow microneedles as the μ RC is ideal. Table 2
 statistically compares the intensity/volume slopes between reflective wells of different
 viewing window sizes, showing that there is a significant increase in the
 25 intensity/volumes slopes, as the μ RC width decreases. The intensity/volume slope
 ratio is the slope for the smaller window over the slope for the next larger window.

30

1

Table 2

5

| μ RC Square Widths Being Compared (μ m) | P value from T-test between Intensity/Volume Slopes μ RCs being compared | Ratio of Intensity/Volume Slopes for Smaller μ RC Widths Verses Wider μ RC Widths |
|--|--|---|
| 500/750 | 0.004 | 1.89 |
| 400/500 | 0.04 | 1.98 |
| 300/400 | 0.28 | 1.59 |
| 250/300 | 0.89 | 1.11 |
| 200/250 | 0.81 | 1.28 |
| 150/200 | 0.91 | 0.73 |

10

The increase in intensity/volume is only significant down to wells that are 400 μ m wide. For smaller μ RC widths (150-300 μ m), the non-reflective substrates show little correlation between average CCD counts and sample volumes ($R^2 < 0.25$, where R is the reflectivity). The Ti/Cr reflective substrates still show some correlation ($R^2 > 0.25$ for μ RCs 250 μ m wide and wider) between intensity and volume. However, as the μ RC square width decreases (250 μ m wide and smaller), there is little difference in signal intensity values for different etch depths. The error in the signals for wells 200 μ m wide and smaller is large enough to overlap with the background intensity. This suggests that the wells with windows smaller than 200 μ m wide would not produce signals of a discernable intensity, if they are only 250 μ m deep. However, narrower and deeper μ RCs such as microneedles, which hold a larger sample volume, would produce a stronger useable signal.

15

The present invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

25

What is claimed is:

30

- 1 1. An active needle device, comprising:
 a hollow non-silicon elongated shaft defining at least one channel and
 having a proximal end and a distal end;
 at least one input port at the proximal end of the elongated shaft and at
5 least one output port at the distal end, the channel providing communication
 between the at least one input port and the at least one output port; and
 at least one active component in the device.
2. The needle device of claim 1, wherein the elongated shaft is configured
 to form a macroneedle.
- 10 3. The needle device of claim 1, wherein the elongated shaft is configured
 to form a microneedle.
4. The needle device of claim 1, wherein the active component comprises
 one or more components selected from the group consisting of electronic components,
 electromechanical components, mechanical components, biochemical components,
15 and combinations thereof.
5. The needle device of claim 1, wherein the active component is selected
 from the group consisting of a sensor, an actuator, and combinations thereof.
6. The needle device of claim 5, wherein the sensor comprises a
 biosensor.
- 20 7. The needle device of claim 6, wherein the biosensor is a
 bioluminescence-based biosensor.
8. The needle device of claim 5, wherein the actuator comprises a
 micropump or microvalve.
9. The needle device of claim 1, wherein the active component comprises
25 an integrated circuit chip.
10. The needle device of claim 1, wherein the active component comprises
 an optical detector.
11. The needle device of claim 1, wherein the active component comprises
 a transducer component.
- 30 12. The needle device of claim 1, wherein the active component facilitates
 taking one or more measurements selected from the group consisting of biochemical
 measurements, optical measurements, electrical measurements, physical

- 1 measurements, and combinations thereof.
13. An active microneedle device, comprising:
a hollow non-silicon elongated shaft defining at least one microchannel
and having a proximal end and a distal end;
5 at least one input port at the proximal end of the elongated shaft and at
least one output port at the distal end, the microchannel providing
communication between the at least one input port and the at least one output
port; and
at least one active component located in the elongated shaft.
- 10 14. The microneedle device of claim 13, wherein the elongated shaft has a
plurality of input ports.
- 15 15. The microneedle device of claim 13, wherein the elongated shaft has a
plurality of output ports.
16. The microneedle array device of claim 13, wherein the elongated shaft
is supported on a substrate.
17. The microneedle device of claim 13, wherein the active component
comprises one or more components selected from the group consisting of electronic
components, electromechanical components, mechanical components, biochemical
components, and combinations thereof.
- 20 18. The microneedle device of claim 13, wherein the active component
comprises a biosensor.
19. The microneedle device of claim 18, wherein the biosensor is a
bioluminescence-based biosensor.
- 25 20. The microneedle device of claim 13, wherein the active component
comprises a transducer component.
21. The microneedle device of claim 13, wherein the elongated shaft has at
least one additional microchannel therein.
22. An active microneedle array device, comprising:
a substrate having a substantially planar surface;
30 a plurality of hollow non-silicon microneedles on the planar
surface of the substrate, each of the microneedles having at least one
microchannel that provides communication between at least one input

1 port at a proximal end of each of the microneedles and at least one
output port at an opposite distal end that extends beyond an edge of the
substrate; and

5 at least one active component located in one or more of the
microneedles.

23. The microneedle array device of claim 22, wherein the microneedles
comprise a two dimensional array.

24. The microneedle array device of claim 22, wherein the microneedles
comprise a three dimensional array.

10 25. The microneedle array device of claim 22, wherein the microchannel
in each of the microneedles has a cross-sectional area in the range from about $25 \mu\text{m}^2$
to about $5000 \mu\text{m}^2$.

26. The microneedle array device of claim 22, wherein the microneedles
15 are composed of a metal material selected from the group consisting of nickel, copper,
gold, silver, platinum, palladium, titanium, chromium, and alloys or combinations
thereof.

27. The microneedle array device of claim 22, further comprising a
coupling channel member that provides fluid communication between the
20 microneedles.

28. The microneedle array device of claim 22, further comprising a pair of
structural support members that mechanically interconnect the microneedles and that
precisely control penetration depth of the microneedles

29. The microneedle array device of claim 22, wherein the microneedles
25 have a plurality of input ports.

30. The microneedle array device of claim 22, wherein the microneedles
have a plurality of output ports.

31. The microneedle array device of claim 22, wherein the active
component comprises one or more components selected from the group consisting of
30 electronic components, electromechanical components, mechanical components,
biochemical components, and combinations thereof.

32. The microneedle array device of claim 22, wherein the active
component comprises a biosensor.

1 33. The microneedle array device of claim 32, wherein the biosensor is a bioluminescence-based biosensor.

 34. The microneedle array device of claim 22, wherein the active component comprises a transducer component.

5 35. The microneedle array device of claim 22, wherein each of the microneedles have at least one additional microchannel therein.

 36. An active microneedle array device, comprising:

 a plurality of hollow non-silicon microneedles each having at least one microchannel that provides communication between at least one input port at a proximal end of each of the microneedles and at least one output port at an opposite distal end;

 at least one structural support member that interconnects the microneedles; and

 at least one active component located in one or more of the microneedles.

 37. The microneedle array device of claim 36, wherein the microneedles comprise a two dimensional array.

 38. The microneedle array device of claim 36, wherein the microneedles comprise a three dimensional array.

20 39. The microneedle array device of claim 36, further comprising a coupling channel member that provides fluid communication between the microneedles.

 40. The microneedle array device of claim 36, wherein the microneedles have a plurality of input ports.

25 41. The microneedle array device of claim 36, wherein the microneedles have a plurality of output ports.

 42. The microneedle array device of claim 36, wherein the active component comprises one or more components selected from the group consisting of electronic components, electromechanical components, mechanical components, biochemical components, and combinations thereof.

30 43. The microneedle array device of claim 36, wherein the active component comprises a biosensor.

1 44. The microneedle array device of claim 43, wherein the biosensor is a bioluminescence-based biosensor.

 45. The microneedle array device of claim 36, wherein the active component comprises a transducer component.

5 46. The microneedle array device of claim 36, wherein each of the microneedles has at least one additional microchannel therein.

 47. An active needle array device, comprising:

 a plurality of hollow non-silicon needles each having at least one channel that provides communication between at least one input
10 port at a proximal end of each of the needles and at least one output port at an opposite distal end; and

 at least one active component located in one or more of the needles.

 48. The needle array device of claim 47, further comprising a coupling
15 channel member that provides fluid communication between the needles.

 49. The needle array device of claim 47, wherein the needles have a plurality of input ports.

 50. The needle array device of claim 47, wherein the needles have a plurality of output ports.

20 51. The needle array device of claim 47, wherein the active component comprises one or more components selected from the group consisting of electronic components, electromechanical components, mechanical components, biochemical components, and combinations thereof.

 52. The needle array device of claim 47, wherein the active component
25 comprises a biosensor.

 53. The needle array device of claim 52, wherein the biosensor is a bioluminescence-based biosensor.

 54. The needle array device of claim 47, wherein the active component comprises a transducer component.

30 55. The needle array device of claim 47, wherein each of the needles has at least one additional channel therein.

 56. The needle array device of claim 47, wherein each of the needles is a

1 macroneedle.

57. The needle array device of claim 47, wherein each of the needles is a microneedle.

58. An active needle device, comprising:

5 a hollow silicon elongated shaft defining at least one channel and having a proximal end and a distal end;

at least one input port at the proximal end of the elongated shaft and at least one output port at the distal end, the channel providing communication between the at least one input port and the at least one output port; and

10 at least one active component in the device, the active component comprising a bioluminescence-based biosensor.

59. An active microneedle device, comprising:

a hollow silicon elongated shaft defining at least one microchannel and having a proximal end and a distal end;

15 at least one input port at the proximal end of the elongated shaft and at least one output port at the distal end, the microchannel providing communication between the at least one input port and the at least one output port; and

20 at least one active component located in the elongated shaft, the active component comprising a bioluminescence-based biosensor.

60. An active microneedle array device, comprising:

a substrate having a substantially planar surface;

25 a plurality of hollow silicon microneedles on the planar surface of the substrate, each of the microneedles having at least one microchannel that provides communication between at least one input port at a proximal end of each of the microneedles and at least one output port at an opposite distal end that extends beyond an edge of the substrate; and

30 at least one active component located in one or more of the microneedles, the active component comprising a bioluminescence-based biosensor.

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61. An active microneedle array device, comprising:

5

a plurality of hollow silicon microneedles each having at least one microchannel that provides communication between at least one input port at a proximal end of each of the microneedles and at least one output port at an opposite distal end;

at least one structural support member that interconnects the microneedles; and

10

at least one active component located in one or more of the microneedles, the active component comprising a bioluminescence-based biosensor.

62. An active needle array device, comprising:

15

a plurality of hollow silicon needles each having at least one channel that provides communication between at least one input port at a proximal end of each of the needles and at least one output port at an opposite distal end; and

at least one active component located in one or more of the needles, the active component comprising a bioluminescence-based biosensor.

20

63. The needle device of claim 1, further comprising a reflective surface in the channel.

64. The needle device of claim 58, further comprising a reflective surface in the channel.

25

65. The needle device of claim 47, wherein the needles have varying lengths.

66. The needle device of claim 62, wherein the needles have varying lengths.

30

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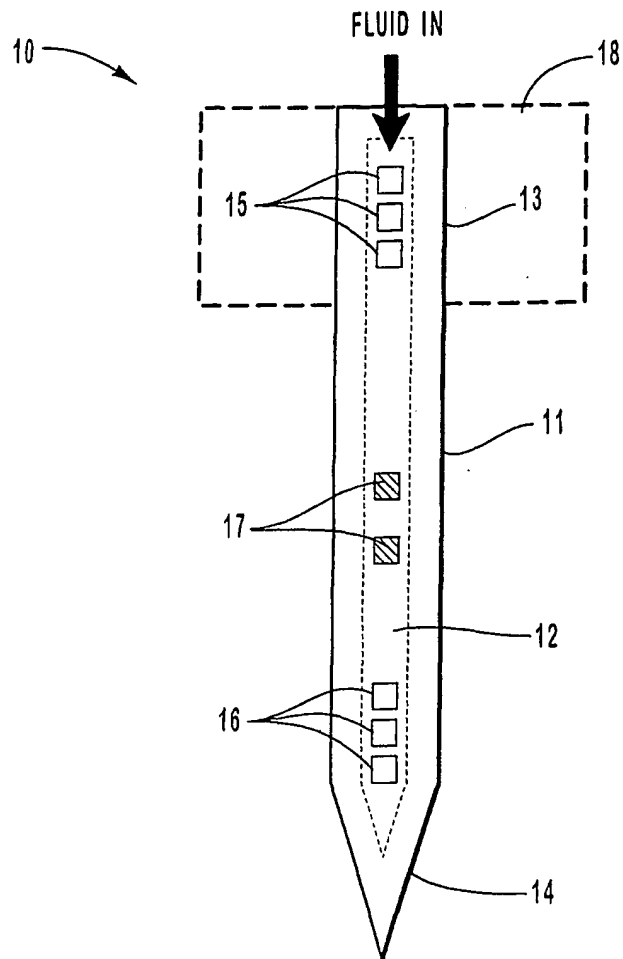


FIG. 1A

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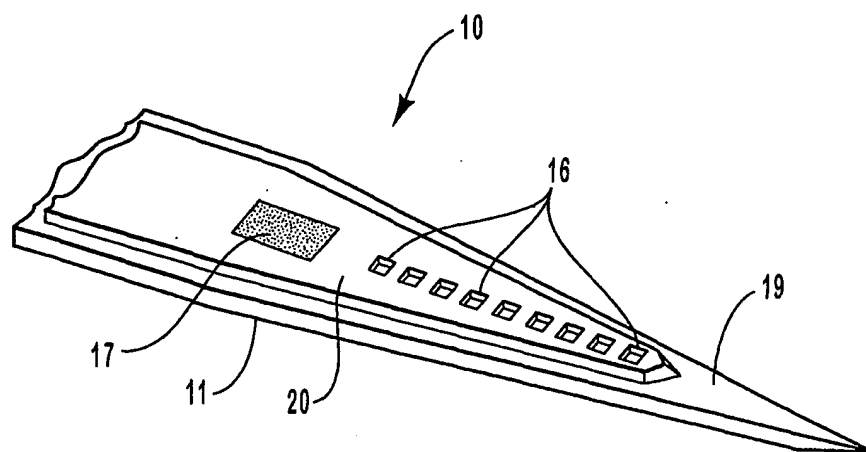


FIG. 1B

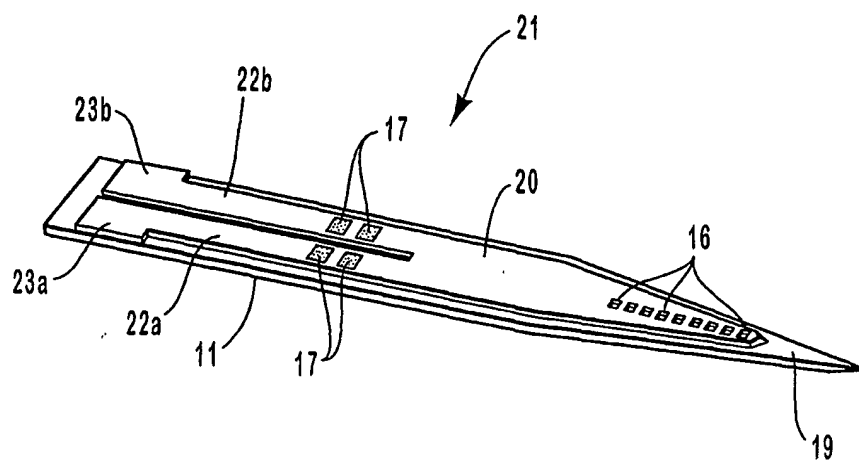


FIG. 2A

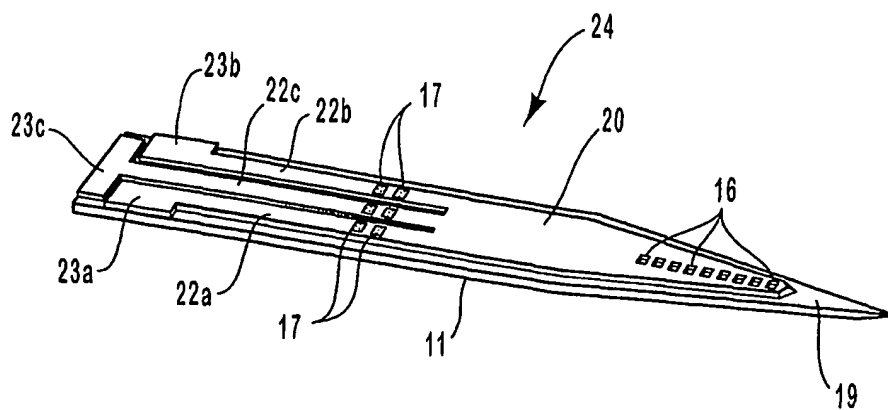
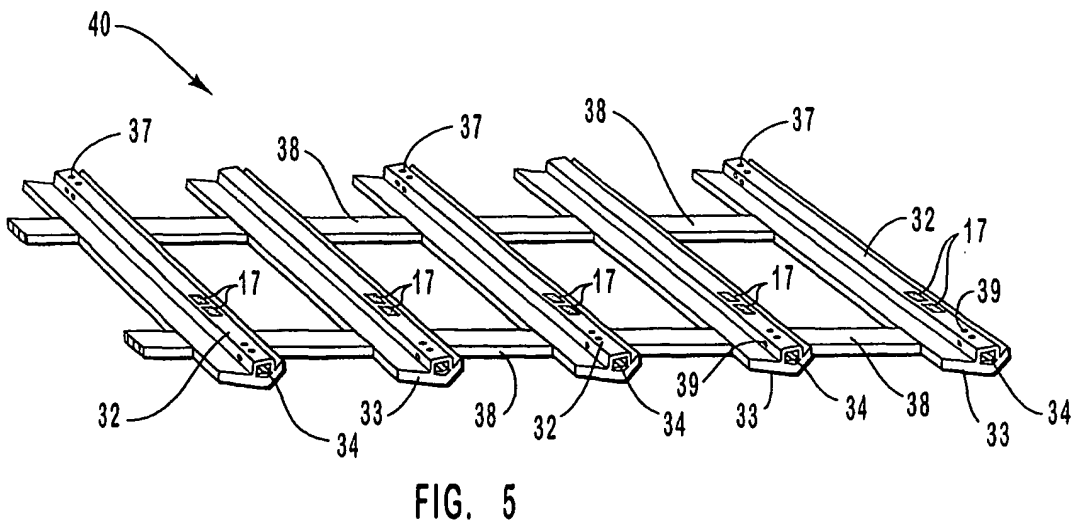
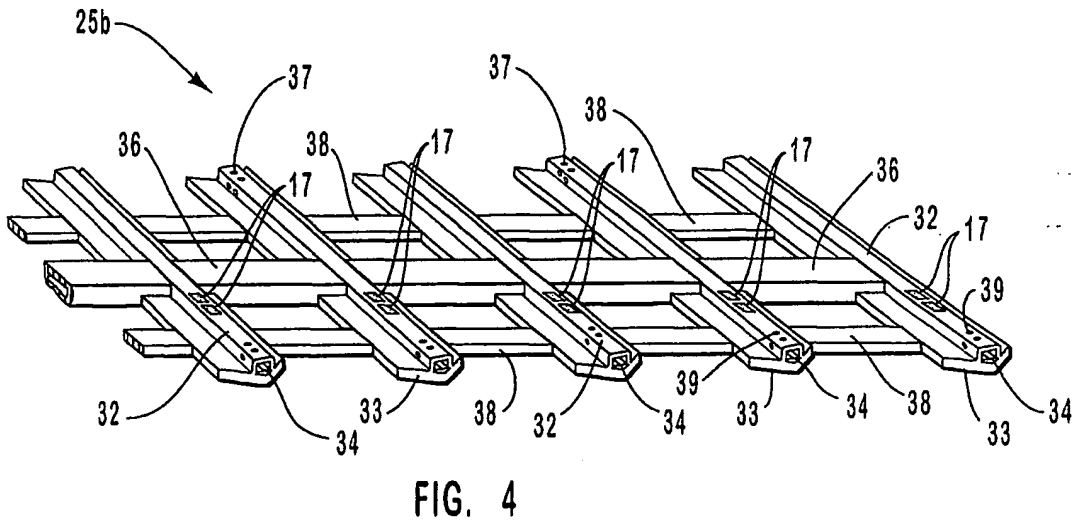
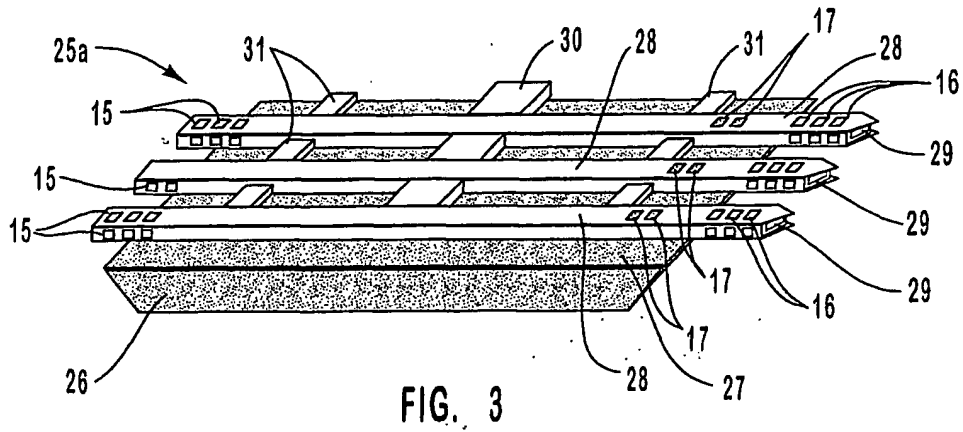


FIG. 2B

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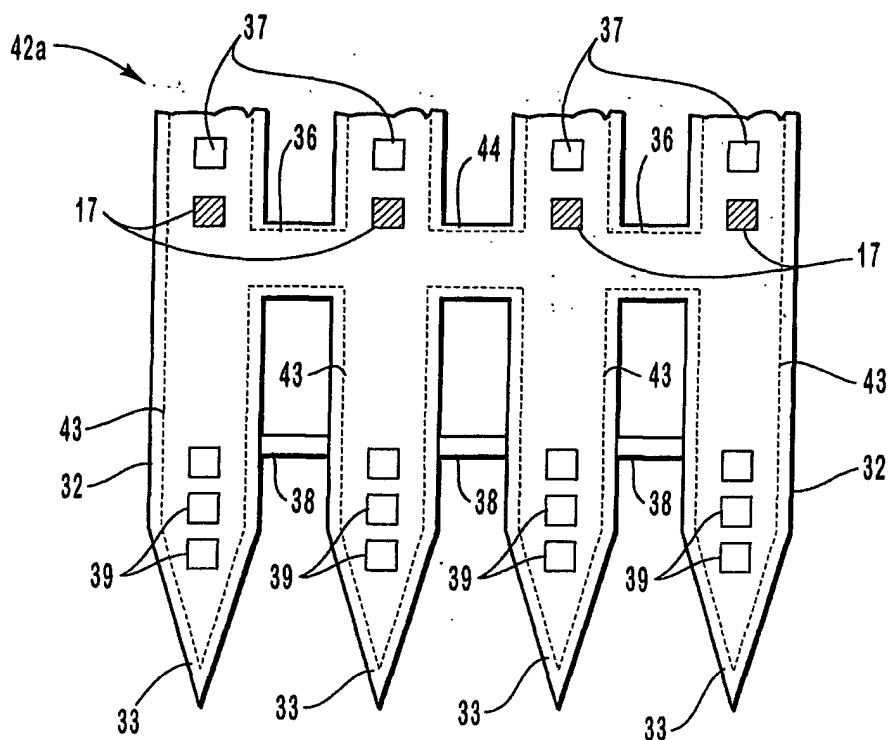


FIG. 6A

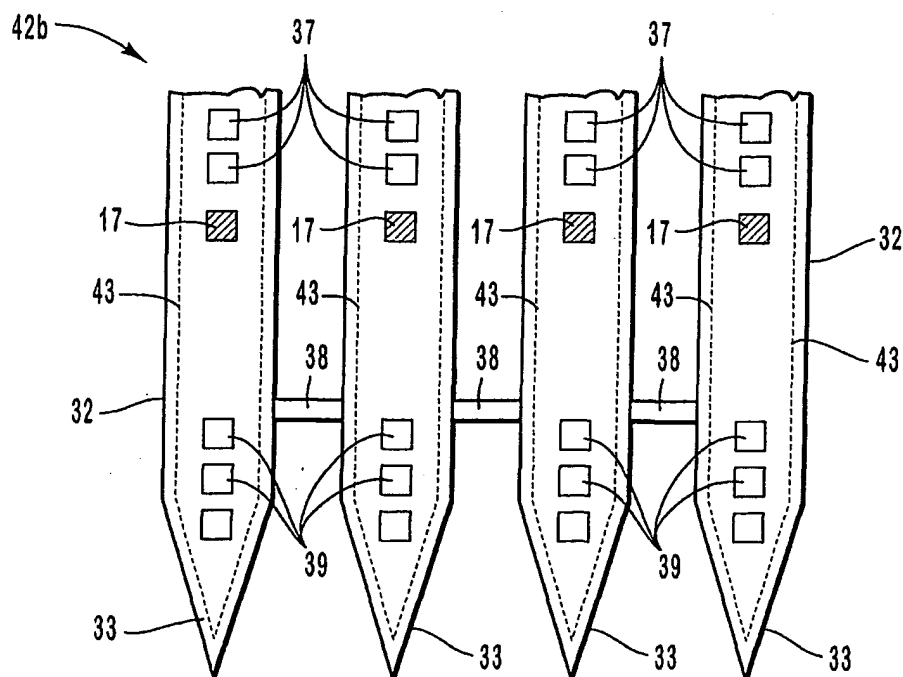


FIG. 6B

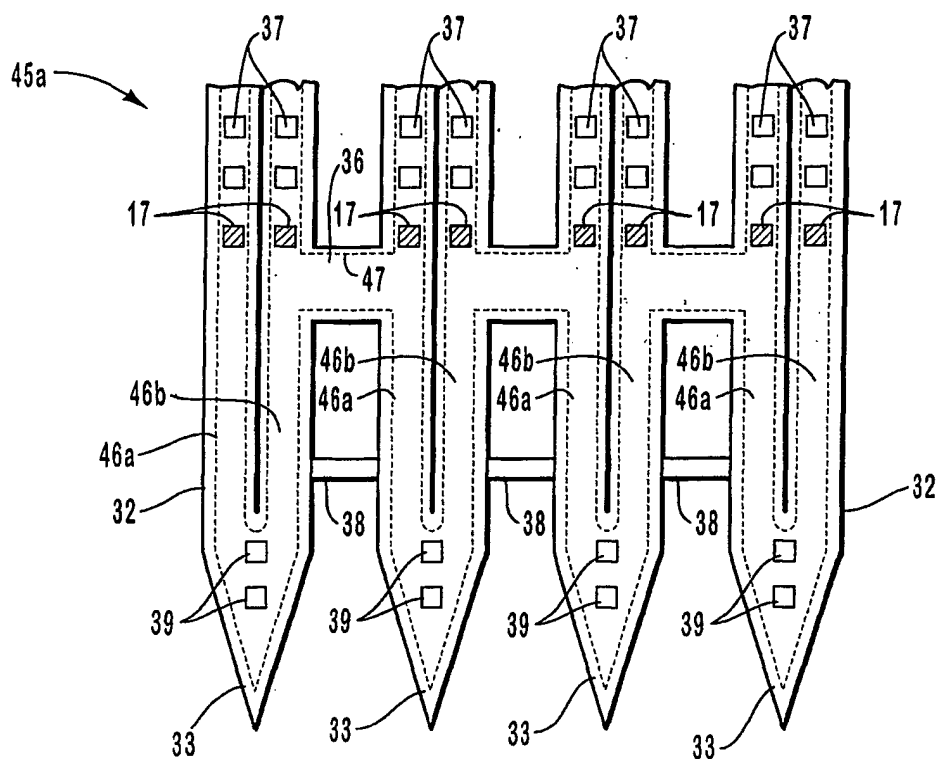


FIG. 7A

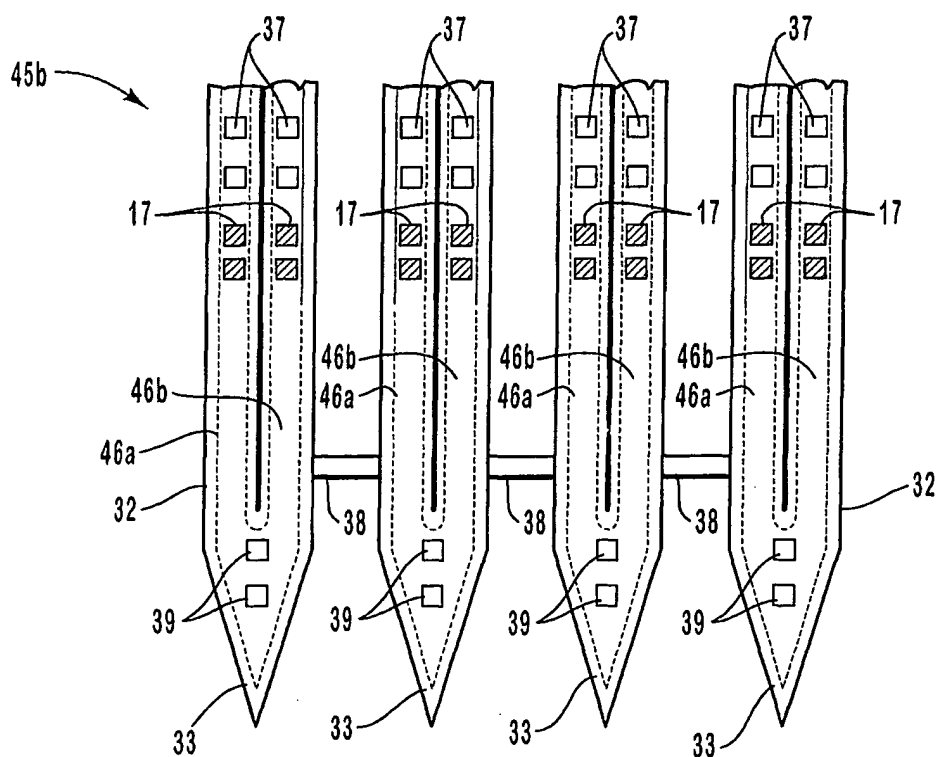


FIG. 7B

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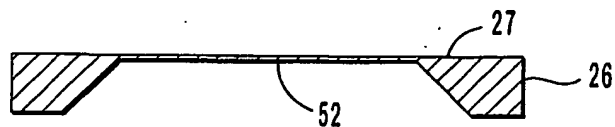


FIG. 8A

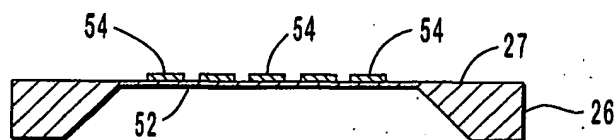


FIG. 8B

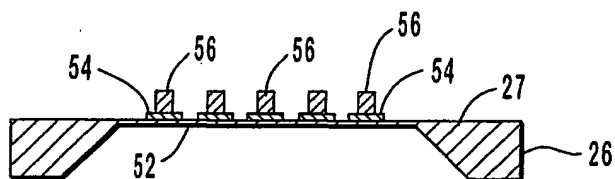


FIG. 8C

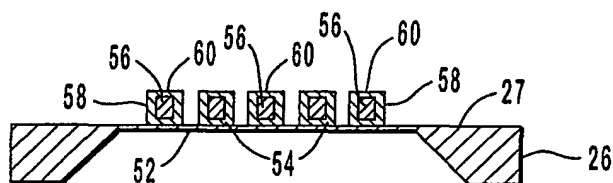


FIG. 8D

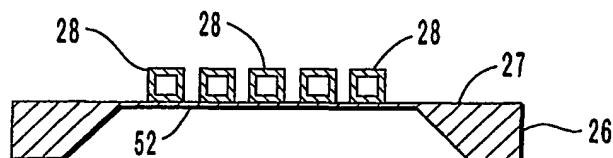


FIG. 8E

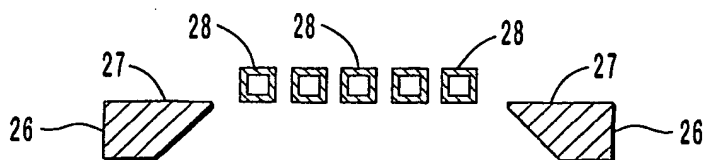


FIG. 8F

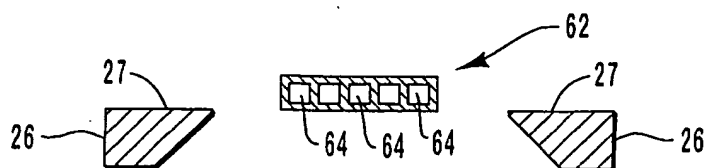
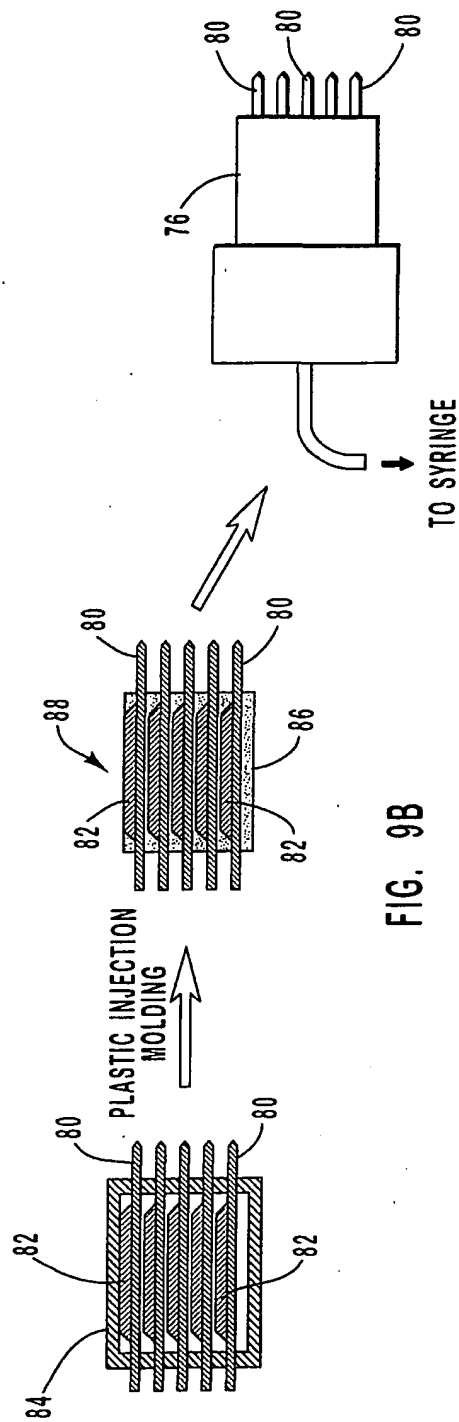
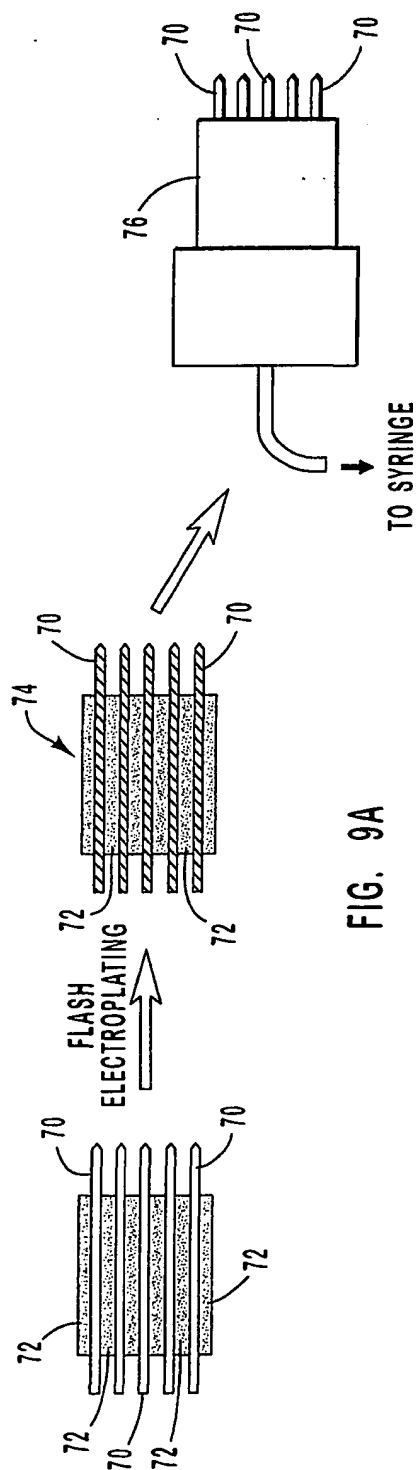


FIG. 8G



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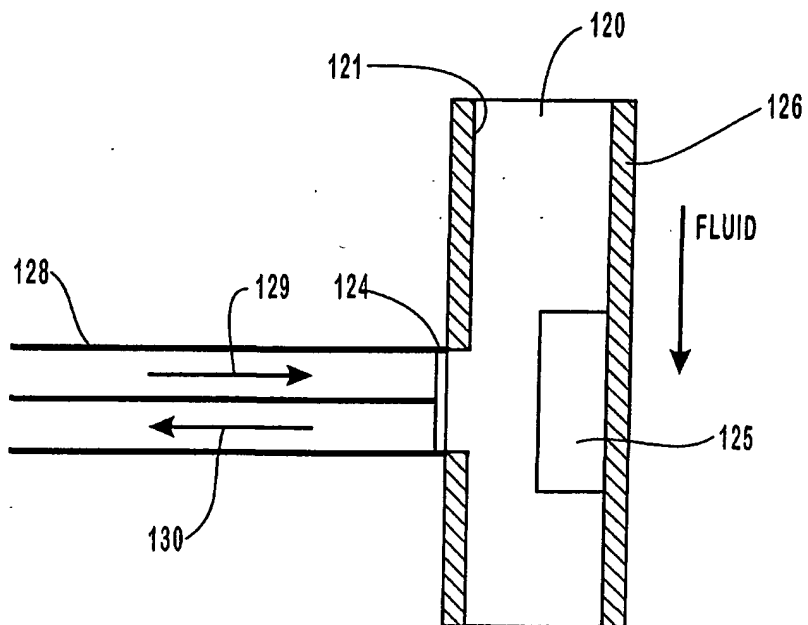


FIG. 10

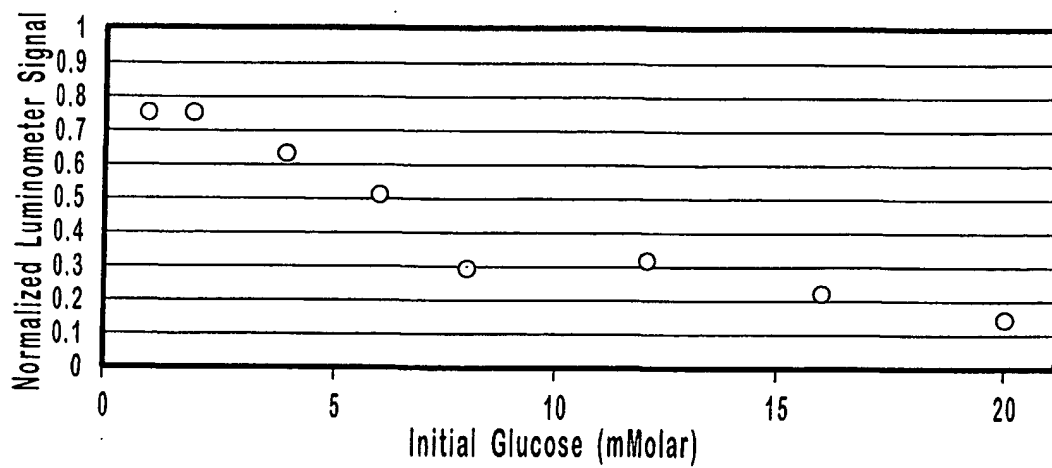


FIG. 11

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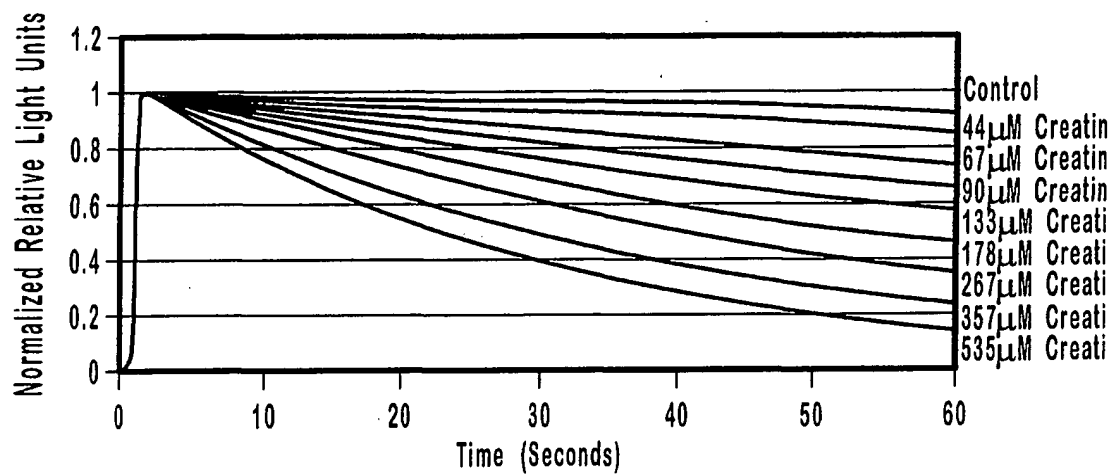


FIG. 12

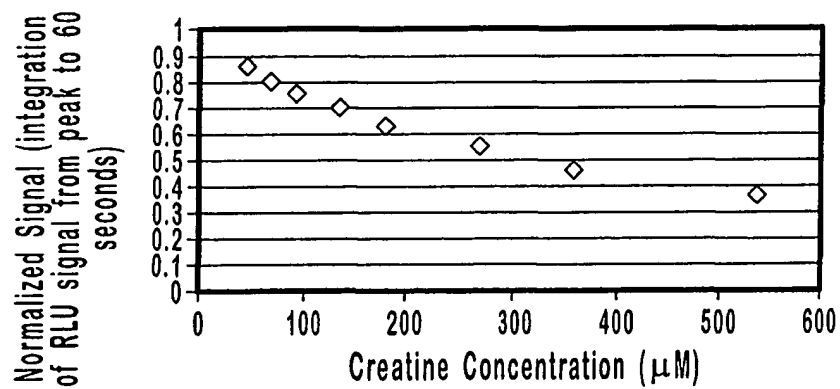


FIG. 13

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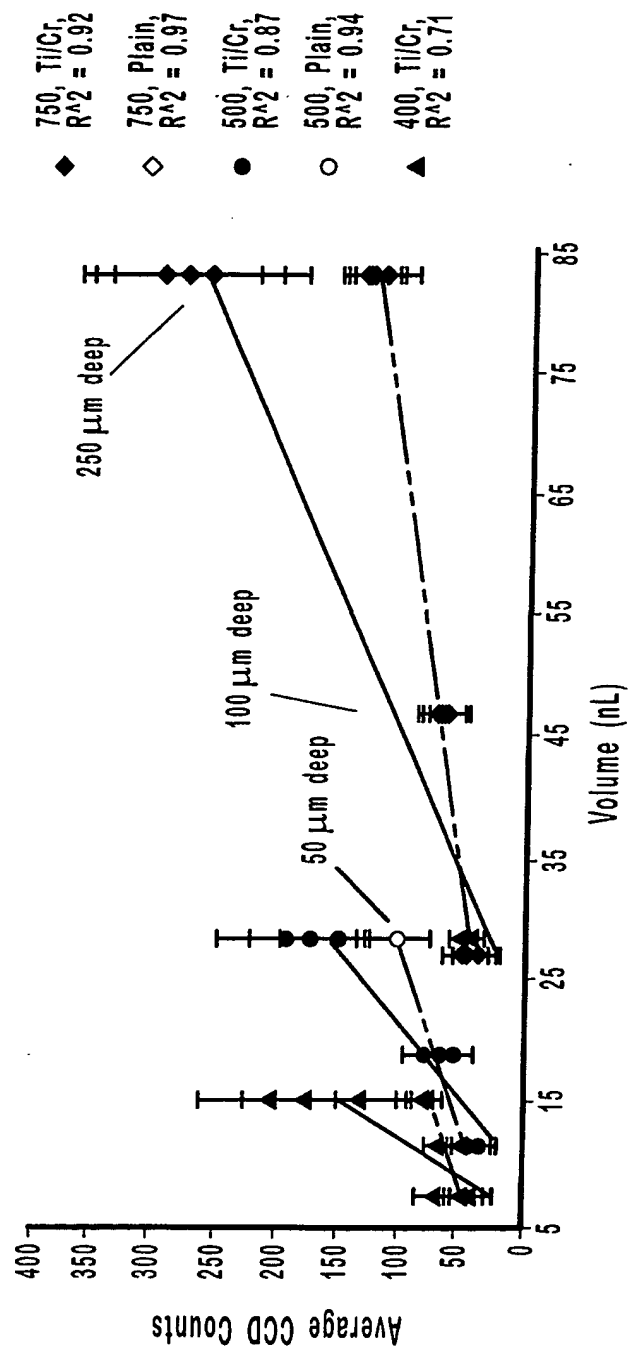


FIG. 14

INTERNATIONAL SEARCH REPORT

 International application No.
 PCT/US01/17838

| A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : A61M 5/32 US CL : 604/272 According to International Patent Classification (IPC) or to both national classification and IPC | | |
|---|--|--|
| B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 604/21, 05, 00, 503, 506, 264, 272-273, 600/222-224, 181 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | US 5,484,399 A (DIRESTA et al) 16 January 1996, entire document. | 1, 2, 4, 5, 8, 12, 63 |
| X | US 6,009,347 A (HOFMANN) 28 December 1999, entire document. | 1, 2, 4, 5, 9, 12, 63 |
| A | US 5,591,139 A (LIN et al) 7 January 1997, entire document. | 22-57, 65 |
| A | US 5,312,456 A (REED et al) 17 May 1994, entire document. | 22-57, 65 |
| <input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex. | | |
| * Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "A" document member of the same patent family | |
| Date of the actual completion of the international search 24 JULY 2001 | | Date of mailing of the international search report 14 AUG 2001 |
| Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230 | | Authorized officer SHARON KENNEDY Telephone No. (703) 305-0154 |